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TITLE OF THE INVENTION (280 characters max)

NEAR-INFRARED IMAGING AGENTS, METHOD AND USE THEREOF

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ENCLOSED APPLICATION PARTS *(check all that apply)*

- ☒ Specification (Including Any Claims and Abstract) - 26 pages
- ☐ Drawings - sheets
- ☒ Other (specify): Application Data Sheet

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Date: August 18, 2003

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Near-infrared imaging agents, method and use thereof

Summary of the Invention

The invention relates to novel near-infrared imaging agents and the use of said agents in a method of labeling amyloid plaques in the brain. In particular, agents of the invention are useful in identifying amyloid formation and/or accumulation in neurological and vascular diseases such as Alzheimer's disease.

Background of the Invention

Alzheimer's disease affects 8% of the population over 65, and at least 35% of those above the age of 80. No current method allows a definitive diagnosis of Alzheimer's disease before autopsy and clinicians can only make a diagnostic of probable Alzheimer's disease by comparing the results of several tests and observations leading to a diagnostic accuracy of about 80 to 90%. The identification of an imaging agent with enough specificity and sensitivity for Alzheimer's disease hallmarks would allow major advances in the way Alzheimer's disease is diagnosed.

Alzheimer's disease is a neurodegenerative disease of the brain characterized by dementia, cognitive impairment, and memory loss. The beta amyloid peptides Ab1-40 and Ab1-42 are major metabolites of the amyloid precursor protein and are found in senile plaques and cerebrovascular amyloid deposits in affected individuals. Formation and accumulation of beta amyloid peptides aggregates in the brain are considered to be critical factors in the development and progression of Alzheimer's disease. Another hallmark of the disease is hyperphosphorylation of the microtubule-associated protein tau with subsequent formation of neurofibrillary tangles.

Currently, the only definitive confirmation of Alzheimer's disease is by postmortem histopathological examination of amyloid deposits and neurofibrillary tangles in the brain. Early appraisal of clinical symptoms for diagnosis is often difficult and unreliable. Therefore, there is a need for in vivo imaging agents which can specifically demonstrate the location and density of amyloid plaques and neurofibrillary tangles in the living brain. Such agents would be useful diagnostic tools for early detection and monitoring of disease progression, as well as for evaluating the effectiveness of treatments of Alzheimer's disease.

Neurofibrillary tangles are cytoskeletal elements composed of aggregates of hyper-phosphorylated tau proteins assembled into periodic restricted amyloid fibres in paired helical filaments. The major component of amyloid plaques is a 39-43 amino acid long beta-amyloid peptide that is generated from the cleavage of a larger amyloid precursor protein. Except for diffuse plaques formed almost exclusively of beta-amyloid peptides, amyloid plaques are complex lesions containing numerous associated cellular products. Deposits of beta-amyloid occur very early in the disease process long before the clinical symptoms develop.

The direct imaging of amyloid deposits in vivo is difficult as the deposits have many of the same physical properties (e.g. density and water content) as normal tissues. However, processes for irradiation and imaging of biological tissues with light of the wavelength range from 600 to 1000 nm in the near-infrared (NIR) region are available.

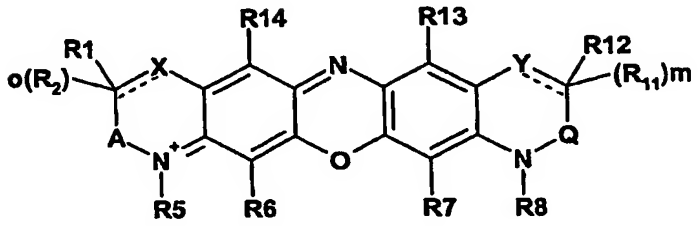
In fluorescence imaging, the energy from an external source of light, e.g. a laser is absorbed and almost immediately re-emitted at a longer wavelength of lower energy. Since biological tissue has a relatively high permeability for long-wave light of the 600 to 1000 nm spectral region, both the detection of non-absorbed radiation in the form of a transmission visualization and the re-emitted fluorescence radiation can provide tissue-specific data. Imaging of deeper tissues (that is, in the range of centimeters) is accomplished by using NIR light in combination with NIR fluorochromes. Near-infrared fluorescence imaging has the advantage of minimizing tissue autofluorescence thus improving target/background ratios.

Suitable agents for in vivo brain imaging should be able to cross the blood-brain barrier to enter the brain in sufficient amounts to be detectable by near-infrared radiation, be of low molecular weight and lipophilic. Further, they should have a high affinity and specific binding to amyloid plaques without being rapidly degraded.

Description of the Invention

The present invention provides novel imaging agents that can be used in near-infrared imaging.

In one aspect of the invention, compounds of formula I are provided in free base or acid addition salt form



X, Y = C or Heteroatom

X and Y are not simultaneously C;

m, o = 0 or 1, with the proviso that

if m is 0 then the dotted line between Y and the neighbouring C atom represents a bond,

if m is 1 then the dotted line between Y and the neighbouring C atom is absent,

if o is 0 then the dotted line between X and the neighbouring C atom represents a bond,

if o is 1 then the dotted line between X and the neighbouring C atom is absent;

wherein A represents $(CR_3R_4)_p$ and Q represents $(CR_9R_{10})_n$,

n, p = 0 or 1

R6, R7, R13, R14 = H, Hal, (C_{1-4})alkyl, $SO_2(C_{1-4})$alkyl, SO_3H, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy, OH, $NR_{15}R_{16}$

R1, R_2, R3, R4, R9, R10, R_{11}, R12 = H, (C_{1-4})alkyl, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy

or when X = C; R1, R_2 can also be OH, $NR_{15}R_{16}$

or when Y = C; R_{11}, R12 can also be OH, $NR_{15}R_{16}$

R5, R8, R15, R16 = H, (C_{1-4})alkyl, (C_{1-4})alkoxy, (C_{1-4})alkyl$COOR_{17}$, (C_{1-4})alkyl(reactive group)* * as defined in Table 1

and

R17 = H, (C_{1-4})alkyl.

In a preferred aspect of the invention, compounds of formula I are provided in free base or acid addition salt form wherein X is O,S or C and Y is O, S or C, provided that X and Y are not both C.

Above and elsewhere in the present description the following terms have the following meanings:
Hal or halogen denote I, Br, Cl or F.

Organic radicals or compounds can be branched or unbranched.

An "alkyl group" is branched or unbranched and contains 1 to 4 carbon atoms. Alkyl represents, for example, methyl, ethyl, propyl, butyl.

An "alkoxy group" is branched or unbranched and contains 1 to 4 carbon atoms. Alkoxy represents for example methoxy, ethoxy, propoxy, butoxy.

"Alkyl carbonyl" refers to a radical of the formula $-C(O)R_a$ where R_a is a alkyl radical defined above.

In another aspect of the invention, compounds according to formula I are capable of being detected by near-infrared radiation of wavelength 600-1000 nm.

In yet another aspect of the invention, compounds of the invention preferably exhibit the following properties:

(i) specificity for amyloid plaques
(ii) blood-brain barrier penetration
(iii) solubility
(iv) capable of being detected by near-infrared radiation

Specificity of a compound of the invention for amyloid plaques is determined to have occurred when there is a chemical interaction between a compound of the invention and

said amyloid plaque. This chemical association includes: covalent bonds, ionic bonds, hydrophilic-hydrophilic interactions or hydrophobic-hydrophobic interactions.

In one aspect of the invention, a composition is provided comprising a compound of formula I and an acceptable excipient or diluent.

In another aspect of the invention, a composition is provided comprising a compound of formula I wherein X is O,S or C and Y is O, S or C, provided that X and Y are not both C and an acceptable excipient or diluent.

In yet another aspect of the invention, a composition is provided comprising a compound of the invention capable of being detected by near-infrared radiation of wavelength 600 – 1000 nm.

Compositions according to the invention comprise a compound of the invention and are intended to include any and all solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, compatible with application. Except insofar as any conventional media or agent is compatible with the active compound, such media can be used in the compositions of the invention. Supplementary active compounds can also be incorporated into the compositions.

For example, when a composition of the invention is applied to a subject, it is formulated to be compatible with its intended route of application. Examples of routes of application include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid or cyclodextrin; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide.

Compounds or compositions of the invention can be attached to cells or vectors and be delivered to a subject by, for example, intravenous injection, local administration or by stereotactic injection.

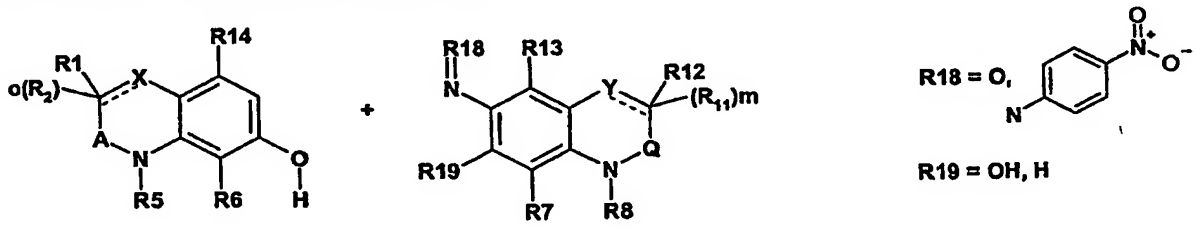
Composition of the present invention may further include one or more of the following components, at the concentrations noted, with the final osmolality adjusted with sodium chloride or potassium chloride to from about 250 milliosmoles (m Osm) to about 330 milliosmoles: K/NaHCO 3 at a concentration of about 5-50 mM; MgCl 2 at a concentration of about 0-88 mM; KCl at a concentration of about 4-104 mM; Na 3 PO 4 at a concentration of about 0-1.5 mM; and CaCl 2 at a concentration of about 0- 0.6 mM. Preferably, the buffer solution is formulated to maintain the pH of the autoretic reagent composition at between about 7 to about 8, most preferably about 7.4, and, accordingly, may include one or more of the following components, in the concentration ranges given, with the final osmolality of from about 280 m Osm to about 300 m Osm: Tris/TEA at a concentration of about 0-150 mM; K 2 Ox/EDTA at a concentration of about 0-121 mM; and KCl/NaCl at a concentration of about 0-155 mM.

Compositions of the present invention may also include certain anions and cations (e. g., alkyl metal chlorides) to facilitate penetration through cell membranes. Non-limiting examples of anions include bicarbonate, chloride borate, barbital, oxalate (Ox), or ethylenediaminetetraacetic acid (EDTA). It is to be noted that not all anions have been found to be effective in promoting penetration across cell membranes. Nonlimiting examples of suitable cations include sodium (e.g., NaCl), potassium (e.g., KCl), trishydroxymethylamino methane (Tris), (Tris[hydroxymethyl]-aminomethane-hydrochloric acid (Tris-HCl), or triethanolamine (TEA).

In a further aspect of the invention, conjugates are provided comprising a compound of the invention covalently linked to a biomolecule through a reactive group. Biomolecules may be selected from the group consisting of: nucleoside, nucleotide, oligonucleotide, nucleic acid, protein, peptide, amino acid, polysaccharide, oligosaccharide, monosaccharide, drug or a small molecule, for example, less than MW 500.

Table 1: Examples of reactive groups for covalent linkages		
Reactive group	Coupled to	product
Activated ester (e.g. NHS)	Amine	amide
Sulfonyl halide	Phenol/alcohol	Sulfonate ester
Sulfonyl halide	Amine	Sulfonamide
Isothiocyanate	Amine	Thiourea
Anhydride	Amine	Amide
Maleimid	Thiol	Thioether
Thiol	Maleimid	Thioether
Haloacetyl	Thiol	Thioether
Hydrazine	Aldehyde	Hydrazone
Amine	Aldehyde	Amine (after reduction)
Amine	Reactive carboxylic acid	Amide
Phosphoramidite	Alcohol	Phosphite esters
Boronates	Glycols	Boronate esters

In another aspect of the invention, a process for the production of compounds of formula I and their salts is provided, comprising:



and recovering the resulting compound of formula I in free base form or in form of an acid addition salt.

Compounds of the invention can produced, for example, as described in Examples 1-5. Working up the reaction mixtures and purification of the compounds thus obtained may be

carried out in accordance to known procedures. Acid addition salts may be produced from the free bases in known manner, and vice-versa.

The invention provides a method of labeling target structures in the brain by applying a compound or composition of the invention. The term "applying" is used in its broadest sense and includes any method of introducing the compounds or compositions of the invention to a subject's body or part of the body. A subject refers to any mammal including, for example, a human, rat, mouse, dog, cat or swine.

The application of a compound or composition of the invention to a subject may be systemically or locally. For example, a compound of the invention may be applied to the subject such that it is delivered throughout the body. Alternatively, a compound of the invention may be applied locally to a specific organ or tissue of interest. This local application may either be in vivo in a living subject or in vitro after a tissue sample has been removed from the body. Examples of the application of a compound or composition of the invention are described in the Examples section.

After a compound or composition of the invention is applied, detection of the compound using near-infrared radiation occurs. The amount of compound needed to be detected can readily be determined by those skilled in the art. Increasing the amount of compound of the invention and comparison to a suitable control can determine the precise dosage of compound needed.

The imaging of amyloid deposits can also be carried out quantitatively so that the amount of amyloid deposits can be determined. For quantitative analysis, the fluorescence images are analyzed on a region of interest basis.

In an aspect of the invention, a method of labeling target structures in the brain is provided, comprising:
(i) applying a composition comprising a compound of formula I in free base or acid addition salt form
(ii) allowing sufficient time for said compound to be chemically associated with the target structure in the brain

(iii) detecting said compound using near-infrared radiation.

In another aspect of the invention, a method of labeling target structures in the brain is provided comprising:

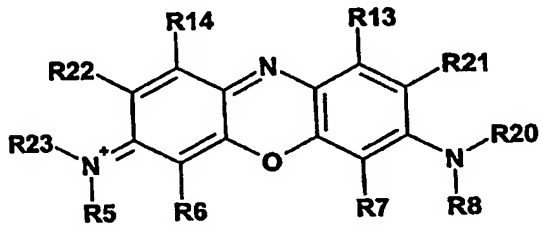
(i) applying a composition comprising a compound of formula I, wherein X is O,S, or C and Y is O,S, or C with the proviso that X and Y are not both C

(ii) allowing sufficient time for said compound to be chemically associated with the target structure in the brain

(iii) detecting said compound using near-infrared radiation.

In yet another aspect of the invention, a method of labeling target structures in the brain is providing comprising:

(i) applying a composition comprising a compound of formula II



formula II

wherein R6, R7, R13, R14 is H, Hal, (C_{1-4})alkyl, $SO_2(C_{1-4})$alkyl, SO_3H, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy, OH, $NR_{15}R_{16}$;

R21, R22 is hydrogen,(C_{1-4}) alkyl, (C_{1-4})alkoxy, phenyl, phenylalkyl, COOH, halogen;

R14 together with R22 can form a saturated or unsaturated C bridge,

R21 together with R22 can form a saturated or unsaturated C bridge;

R5, R8, R20, R23 is hydrogen, ,(C_{1-4}) alkyl, (C_{1-4})alkoxy, polyoxyhydrocarbyl, phenyl, phenylalkyl;

R8 together with R20 can form a saturated or unsaturated C bridge,

R23 together with R5 can form a saturated or unsaturated C bridge,

R22 together with R23 can form a saturated or unsaturated C bridge,

R5 together with R6 can form a saturated or unsaturated C bridge,

R7 together with R8 can form a saturated or unsaturated C bridge,

R20 together with R21 can form a saturated or unsaturated C bridge

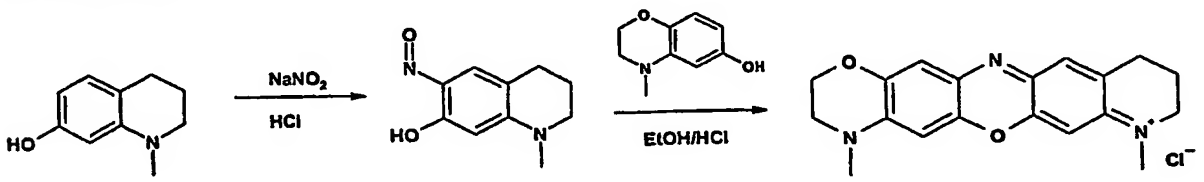
(ii) allowing sufficient time for said compound to be chemically associated with the target structure in the brain
(iii) detecting said compound using near-infrared radiation.

Compounds and compositions of the invention are useful as markers for labeling pathological structures such as amyloid plaques in the brain. This is useful in identifying, diagnosing and preventing diseases involving the formation and/or accumulation of amyloid plaques and for monitoring the effectiveness of therapeutic treatments of diseases involving formation and/or accumulation of amyloid plaques. These diseases include, for example, Alzheimer's disease, Down's Syndrome, memory and cognitive impairment, dementia, amyloid neuropathies, brain inflammation, nerve and brain trauma, vascular amyloidosis, or cerebral haemorrhage with amyloidosis.

In another aspect of the invention, compounds and compositions of the invention are used as near infra-red imaging agents for identifying amyloid plaques in the brain.

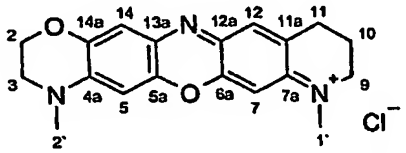
The following examples are illustrative, but not limiting, of the method and compositions of the present invention. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered and obvious to those skilled in the art are within the spirit and scope of the invention.

Example 1: Synthesis of 4,8-Dimethyl-2,3,4,9,10,11-hexahydro-1,6-dioxa-4,13-diaza-8-azonia-pentacen chloride (I)



A solution of 1-methyl-1,2,3,4-tetrahydro-quinolin-7-ol[lit] (20.0 mg, 123 µmol) in water (500 µl) and 2 M HCl (100 µl) was cooled to 0°C and an aqueous solution of sodium nitrite (8.57 mg, 123 µmol) was added. The reaction mixture was stirred at 0°C for 60 min, neutralized

with a saturated solution of $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried with $MgSO_4$ and the solvent was removed under reduced pressure. The obtained nitroso intermediate and 4-methyl-2-H-benz[1,4]oxazin-6-ol[III] (24.5 mg, 148 µmol) were dissolved in a mixture of ethanol (1.00 ml) and 2 M HCl (100 µl) and heated under reflux for 1 h. The solution was concentrated under reduced pressure and the residue was purified by column chromatography (SiO_2, dichloromethane/methanol = 10/3).



yield: 20.0 mg (55.9 µmol, 45 %,), blue crystals

R_f = 0.5 (dichloromethane/methanol = 10/3).

mp.: 245-248 °C

^{1}H NMR (500 MHz): δ = 2.05 (tt, 2 H, ^3J = 6.1 Hz, ^3J = 5.5 Hz, 10-H), 2.92 (t, 2 H, ^3J = 6.1 Hz, 11-H), 3.31 (s, 3 H, 1`-H), 3.33 (s, 3 H, 2`-H), 3.70 (t, 2 H, ^3J = 5.5 Hz, 9-H), 3.78 (t, 2-H, ^3J = 4.9 Hz, 3-H), 4.35 (t, 2 H, ^3J = 4.9 Hz, 2-H), 6.82 (s, 1 H, 7-H), 6.90 (s, 1 H, 5-H), 7.09 (s, 1 H, 11-H), 7.40 (s, 1 H, 12-H).

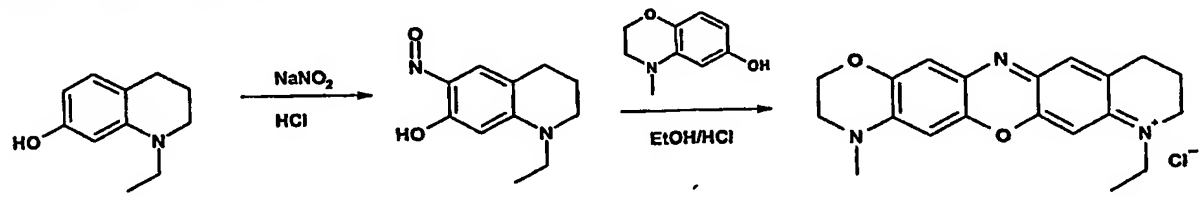
^{13}C NMR (125 MHz): δ = 21.84 (C-10), 28.26 (C-11), 39.05 (C-1`), 41.10 (C-2`), 50.14 (C-9), 53.55 (C-3), 64.74 (C-2), 96.11 (C-7), 96.21 (C-5), 114.8 (C-11), 130.9 (C-12), 131.3 (C-11a), 134.9 (C-13a), 136.5 (C-12a), 147.0 (C-14a, C-5a), 148.6 (C-4a), 149.7 (C-6a), 156.3 (C-7a).

MS (70 eV, EI): m/z (%) = 322.1 (100) $[M]^+$.

HRMS ($C_{19}H_{20}N_3O_2$, M^+): calc. 322.1556

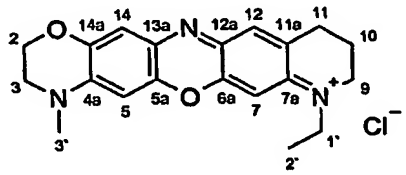
found322.1558

Example 2: Synthesis of 8-Ethyl-4-methyl-2,3,4,9,10,11-hexahydro-1,6-dioxa-4,13-diaza-8-azonia-pentacen chloride (II)



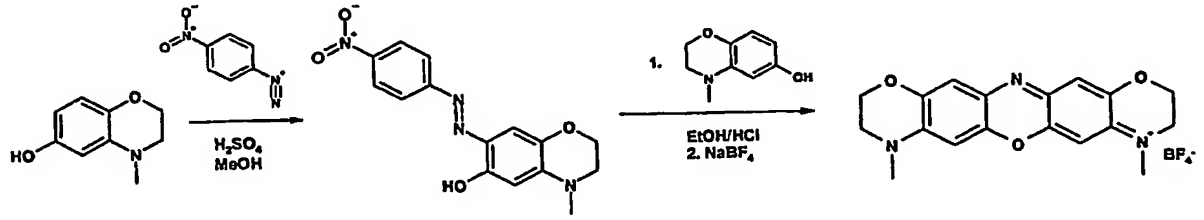
A solution of 1-ethyl-1,2,3,4-tetrahydro-quinolin-7-ol[III] (50.0 mg, 276 µmol) in water (1 ml) and 2 M HCl (250 µl) was cooled to 0°C and a solution of sodium nitrite (20.0 mg, 287 µmol) in water (400 µl) was added. The reaction mixture was stirred at 0°C for 60 min, neutralized

with a saturated solution of $NaHCO_3$ and extracted with ethyl acetate. The organic phase was dried with $MgSO_4$ and the solvent was removed under reduced pressure. The nitroso intermediate (25.0 mg, 121 µmol) and 4-methyl-2-H-benz[1,4]oxazin-6-ol[lit] (20.1 mg, 121 µmol) were dissolved in a mixture of ethanol (750 µl) and 2 M HCl (124 µl) and heated under reflux for 3 h. The solution was concentrated under reduced pressure and the residue was purified by column chromatography (SiO_2, dichloromethane/methanol/water/ acetic acid = 10/10/1/1).



yield: 36.0 mg (96.8 µmol, 80 %), blue crystals.
R_f = 0.5 (dichloromethane/methanol = 10/3).
mp.: 245-247 °C
^{1}H NMR (500 MHz): δ = 1.24 (t, 3 H, ^3J = 6.5 Hz, 2`-H), 1.94 (m, 2 H, 10-H), 2.89 (m, 2 H, 11-H), 3.34 (s, 3 H, 3`-H), 3.67 (m, 2 H, 9-H), 3.73 (m, 2H, 1`-H), 3.79 (m, 2 H, 3-H), 4.34 (m, 2 H, C-2), 6.99 (s, 1 H, 7-H), 7.01 (s, 1 H, 5-H), 7.22 (s, 1 H, 14-H), 7.55 (s, 1 H, 6-H).
^{13}C NMR (125 MHz): δ = 11.40 (C-2`), 20.30 (C-10), 26.77 (C-11), 47.46 (C-1`), 48.63 (C-3), 49.83 (C-9), 63.23 (C-2), 94.69 (C-7), 95.21 (C-5), 113.3 (C-14), 129.5 (C-11a), 129.8 (C-12), 133.2 (C-13a), 134.6 (C-12a), 145.1 (C-5a), 146.7 (C-4a), 148.0 (C-6a), 153.4 (C-7a).
MS (70 eV, EI): m/z (%) = 340.1 (100) $[M]^+$.324 (8).
HRMS ($C_{20}H_{22}N_3O_2$, M^+): calc. 336.1712
found. 336.1712
Log P: 1.9
Log D (pH = 6.8): 1.9, pKa: 5.9

Example 3: 4,8-Dimethyl-3,8,9,10-tetrahydro-2H-1,6,11-trioxa-8,13-diaza-4-azonia-pentacen tetrafluoroborate (III)

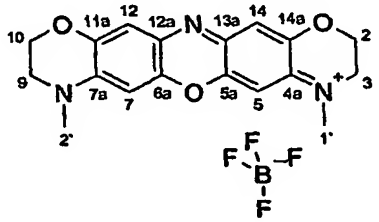


4-Nitro-benzenediazonium tetrafluoroborate (574 mg, 2.42 mmol) was dissolved in 10% H_2SO_4 (400 µl) and was added to a solution of 4-methyl-2-H-benz[1,4]oxazin-6-ol (400 mg,

2.42 mmol) in methanol (2 ml). The reaction mixture was stirred for 30 min at RT, neutralized with 25 % aqueous ammonia and the red precipitate was spun down. The crude diazo intermediate was purified by recrystallization (*n*-butanol).

yield: 720 mg (2.29 mmol, 95 %), red powder.
mp.: 162-165 °C.
^{1}H NMR (500 MHz): δ = 3.09 (s, 3 H, 7'-H), 3.58 (d, 2 H, ^3J = 4.9 Hz, 3-H), 4.21 (d, 2 H, ^3J = 4.9 Hz, 2-H), 5.73 (s, 1 H, 5-H), 6.48 (s, 1 H, 8-H), 7.63 (d, 2 H, ^3J = 9.1 Hz, 2'-H, 6'-H), 8.24 (d, 2 H, ^3J = 9.1 Hz, 3'-H, 5'-H).
^{13}C NMR (125 MHz): δ = 38.80 (C-7'), 47.93 (C-3), 63.61 (C-2), 96.85 (C-5), 110.8 (C-8), 116.3 (C-2', C-6'), 125,6 (C-3', C-5'), 134.2 (C-7), 142.4 (C-8a), 142.8 (C-4'), 147.5 (C-4a), 149.9 (C-1').
MS (70 eV, EI): m/z (%) = 337.1 (6) $[M+Na]^+$, 315.1 (100) $[M+H]^+$, 289.1 (5), 242.3 (10), 177.0 (40), 164.9 (19).
HRMS ($C_{15}H_{14}N_4O_4$, M^+): calc. 315.1088 found315.1088 $[2M+H]^+$: calc. 629.2103 found629.2103

Diazo intermediate (560 mg, 1.78 mmol) and 4-methyl-2-H-benz[1,4]oxazin-6-ol (326 mg, 1.96 mmol) were dissolved in a mixture of ethanol (10 ml) and water (1 ml). After the addition of 32 % HCl (700 µl) the reaction mixture was stirred at 70°C under reflux for 1 h and subsequently the solution was concentrated under reduced pressure. The residue was dissolved in water and treated with a saturated solution of sodium tetrafluoroborate. The precipitate was spun down and purified by column chromatography (SiO_2, dichloromethane/methanol = 10/2).



yield: 480 mg (1.17 mmol, 66 %), blue crystals.
R_f = 0.65 (dichloromethane/methanol = 10/2).
mp.: 235-238 °C.
^{1}H NMR (500 MHz): δ = 3.37 (s, 6 H, 1'-H, 2'-H), 3.80 (t, 4 H, ^3J = 4.8 Hz, 3-H, 9-H), 4.37 (t, 4 H, ^3J = 4.8 Hz, 2-H, 10-H), 7.03 (s, 2 H, 5-H, 7-H), 7.21 (s, 2 H, 12-H, 14-H).
^{13}C NMR (125 MHz): δ = 48.66 (C-3), 63.30 (C-2), 95.10 (C-5, C-7), 112.8 (C-12, C-14), 134.5 (C-12a, C-13a), 145.2 (C-5a, C-6a), 145.7 (C-11a, C-14a), 146.8 (C-4a, C-7a).

MS (70 eV, EI): m/z (%) = 324.2 (100) $[M]^+$, 310 (3).

HRMS ($C_{18}H_{18}N_3O_3$, M^+): calc. 324.1348

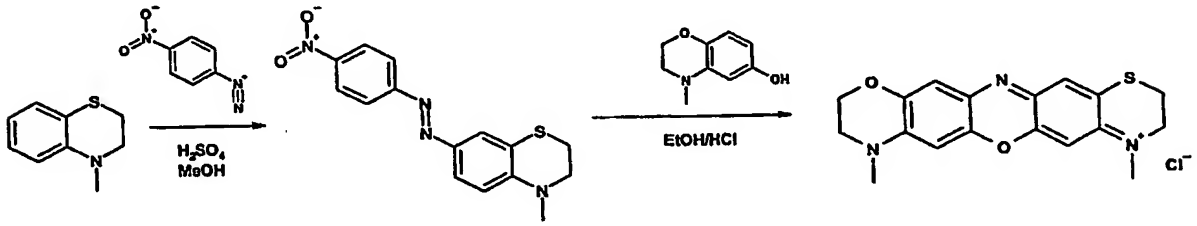
found 324.1349

Log P: 1.7

Log D (pH = 6.8): 1.7

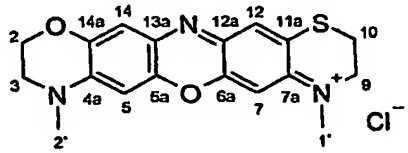
pKa: 5.9

Example 4: 4,8-Dimethyl-2,3,9,10-tetrahydro—4H-1,6-dioxa-11-thia-4,13-diaza-8-azonia-pentacen chloride (IV)



4-Nitro-benzenediazonium tetrafluoroborate (287 mg, 1.21 mmol) was dissolved in 10 % H_2SO_4 (200 µl) and was added to a solution of 4-methyl-3,4-dihydro-2H-benzo[1,4]thiazin (200 mg, 1.21 mmol) in methanol (2 ml). The reaction mixture was stirred for 30 min at RT, neutralized with 25 % aqueous ammonia and the red precipitate was spun down. The crude diazo intermediate was used in the next stage without further purification.

Diazo intermediate (150 mg, 477 µmol) and 4-methyl-2-H-benz[1,4]oxazin-6-ol (119 mg, 716 µmol) were dissolved in a mixture of ethanol (4 ml) and water (400 µl). After the addition of 32 % HCl (187 µl) the reaction mixture was heated at 70°C under reflux for 1 h and subsequently the solution was concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2, dichloromethane/methanol = 10/3).



yield: 15.0 mg, (39.9 µmol, 8 %), blue crystals.

R_f = 0.5 (dichloromethane/methanol = 10/3).

mp.: 250-252 °C

^{1}H NMR (500 MHz): δ = 3.17 (t, 2 H, ^3J = 4.9 Hz, 10-H), 3.37 (s, 3 H, 1`-H), 3.41 (s, 3 H, 2`-H), 3.85 (m, 2 H, 9-H), 4.03 (m, 2 H, 3-H), 4.38 (d, 2 H, ^3J = 4.3 Hz, 2-H), 6.99 (s, 1 H, 7-H), 7.02 (s, 1 H, 5-H), 7.11 (s, 1 H, 14-H), 7.54 (s, 1 H, 12-H).

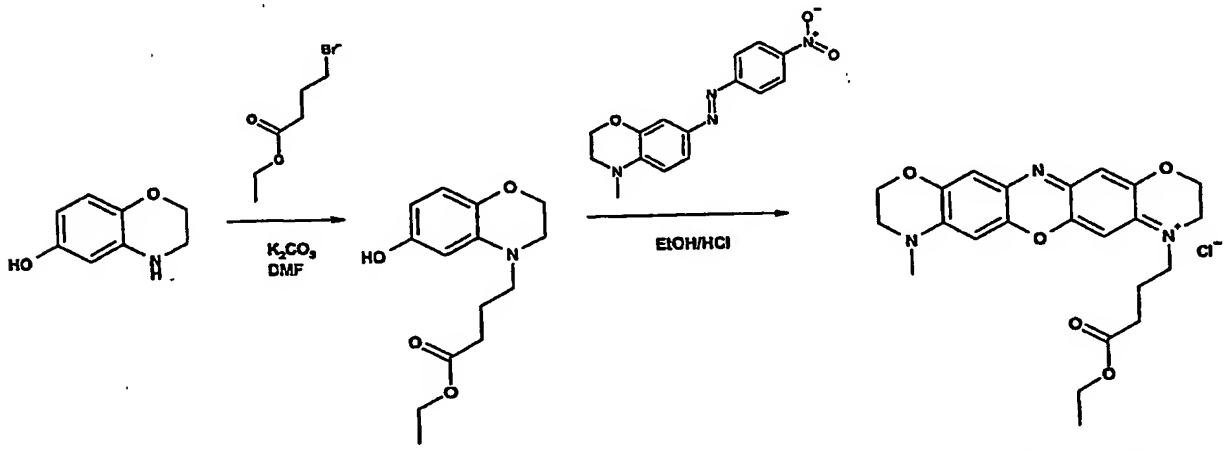
^{13}C NMR (125 MHz): δ = 24.48 (C-10), 40.55 (C-1`), 42.53 (C-2`), 50.37 (C-9), 54.14 (C-3), 64.73 (C-2), 96.52 (C-7), 97.24 (C-5), 114.7 (C-14), 126.4 (C-11a), 128.9 (C-12), 134.8 (C-12a), 147.7 (C-14a), 147.9 (C-5a), 148.5 (C-4a), 149.7 (C-6a), 152.9 (C-7a).

MS (70 eV, EI): m/z (%) = 340.1 (100) $[M]^+$.324 (8).

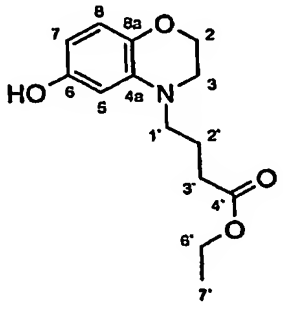
HRMS ($C_{18}H_{18}N_3O_2S$, M^+): calc. 340.1120

found340.1122

Example 5: 8-(3-Ethoxycarbonyl-propyl)-4-methyl-3,8,9,10-tetrahydro-2H-1,6,11-trioxa-8,13-diaza-4-azonia-pentacen chloride (V)



A solution of 3,4-dihydro-2H-1,4-benzoxazin-6-ol (200 mg, 1.32 mmol), potassium carbonate (183 mg, 1.32 mmol) and 4-bromo-butyric acid ethyl ester (217 µl, 1.46 mmol) in anhydrous DMF (1 ml) was stirred at 65°C for 16 h under argon. The reaction mixture was poured into water (20 ml), the aqueous phase was neutralized with 2 M HCl and extracted with ethyl acetate (3 x 50 ml). The combined organic phases were dried with $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, hexane/ethyl acetate = 2/1).



yield: 210 mg (792 µmol, 60 %), colorless oil.

R_f = 0.4 (hexane/ethyl acetate = 2/1).

^1H NMR (500 MHz): δ = 1.17 (t, 3 H, ^3J = 7.4 Hz, 7`-H), 1.76 (dd, 2 H, ^3J = 7.4 Hz, 2´-H),

2.34 (t, 2 H, ^3J = 7.3 Hz, 3'-H), 3.17 (t, 2 H, ^3J = 7.4 Hz, 1'-H), 3.23 (d, 2 H, ^3J = 4.5 Hz, 3-H), 4.05 (m, 4 H, 2-H, 6'-H), 5.89 (dd, 1 H, ^3J = 7.9 Hz, ^4J = 2.4 Hz, 7-H), 6.11 (d, 1 H, ^4J = 2.4 Hz, 5-H), 6.42 (d, 1 H, ^3J = 7.9 Hz, 8-H), 8.62 (s, 1 H, OH).

^{13}C NMR (125 MHz): δ = 14.15 (C-7'), 21.00 (C-2'), 30.96 (C-3'), 46.61 (C-3), 49.35 (C-1'), 59.91 (C-6'), 63.71 (C-2), 99.22 (C-5), 102.7 (C-7), 115.9 (C-8), 134.3 (C-4a), 136.5 (C-8a), 152.0 (C-6), 172.7 (C-4').

MS (70 eV, EI): m/z (%) = 529.3 (8) $[2M-H]^-$, 310.2 (22) $[M+HCOO]^-$, 264.2 (100) $[M-H]^-$, 236.1 (23).

HRMS ($C_{14}H_{19}NO_4$, M^+): calc. 266.1392
found266.1391

Diazo intermediate (150 mg, 565 µmol) and 4-(6-Hydroxy-2,3-dihydro-benzo[1,4]oxazin-4-yl)-butyric acid ethyl ester (213 mg, 678 µmol) were dissolved in a mixture of ethanol (1 ml) and water (100 µl). After the addition of 32 % HCl (222 µl) the reaction mixture was heated at 70°C under reflux for 1 h and the solution was concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, dichloromethane/methanol = 10/2).

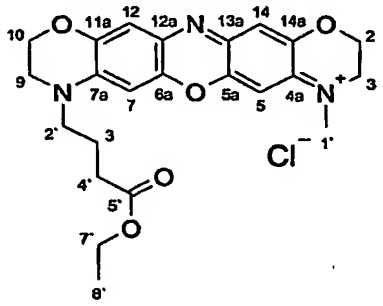
yield: 15.0 mg (32.6 µmol, 6 %), blue crystals.

R_f = 0.7 (dichloromethane/methanol = 10/2).

mp: 242-245 °C.

^{1}H NMR (500 MHz): δ = 1.26 (t, 3 H, ^3J = 6.8 Hz, 8'-H), 2.05 (m, 2 H, 3'-H), 2.51 (t, 2 H, ^3J = 6.7 Hz, 4'-H), 3.71 (t, 2 H, ^3J = 7.4 Hz, 2'-H), 3.79 (m, 4 H, 3-H, 9-H), 4.17 (q, 2 H, 6.8 Hz, 7'-H), 4.35 (m, 2 H, 2-H, 10-H), 6.99 (s, 1 H, 7-H), 7.16 (m, 3 H, 5-H, 12-H, 14-H).

^{13}C NMR (125 MHz): δ = 14.58 (C-8'), 22.35 (C-3'), 31.40 (C-4'), 40.24 (C-1'), 52.69 (C-2'), 61.85 (C-7'), 64.77 (C-2, C-10), 96.30 (C-5, C-7), 114.4 (C-12, C-14), 136.6 (C-12a, C-13a), 147.7 (C-4a, C-5a, C-6a, C-7a, C-11a, C-14a), 174.6 (C-5').



MS (70 eV, EI): m/z (%) = 424.1 (100) $[M]^+$, 410.1 (10).

HRMS ($C_{23}H_{26}N_3O_5$, M^+): calc. 424.1872
found424.1878

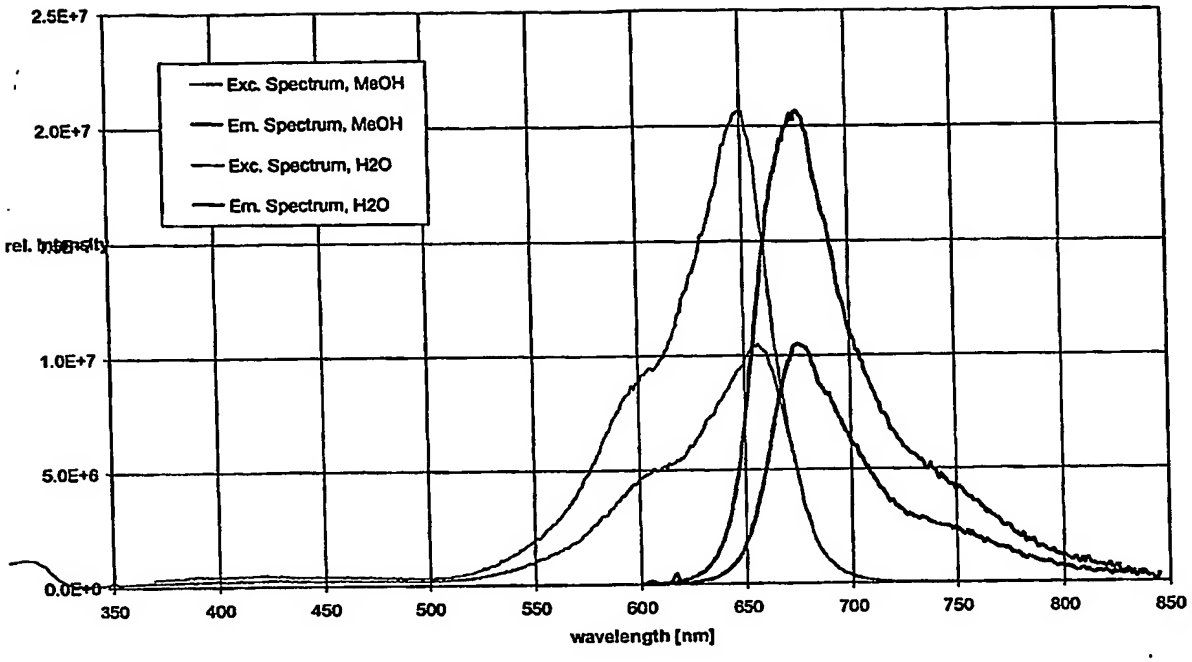
Example 6: UV/VIS and fluorescence properties of compounds of the invention

UV/VIS and fluorescence spectra are recorded and analyzed. For UV/VIS spectra, a Lambda 19 spectrometer from Perkin Elmer equipped with cuvettes of 1.000 cm pathlength is used. The scan rate is 120 nm/min. The fluorescence data are obtained on a Spex Fluorolog (I.S.A.) spectrometer equipped with a cooled R928 detector (Slit 1 mm). Quantum yields are determined by using Cresylviolett (exc. 595 nm, yield = 0.54) as standard.

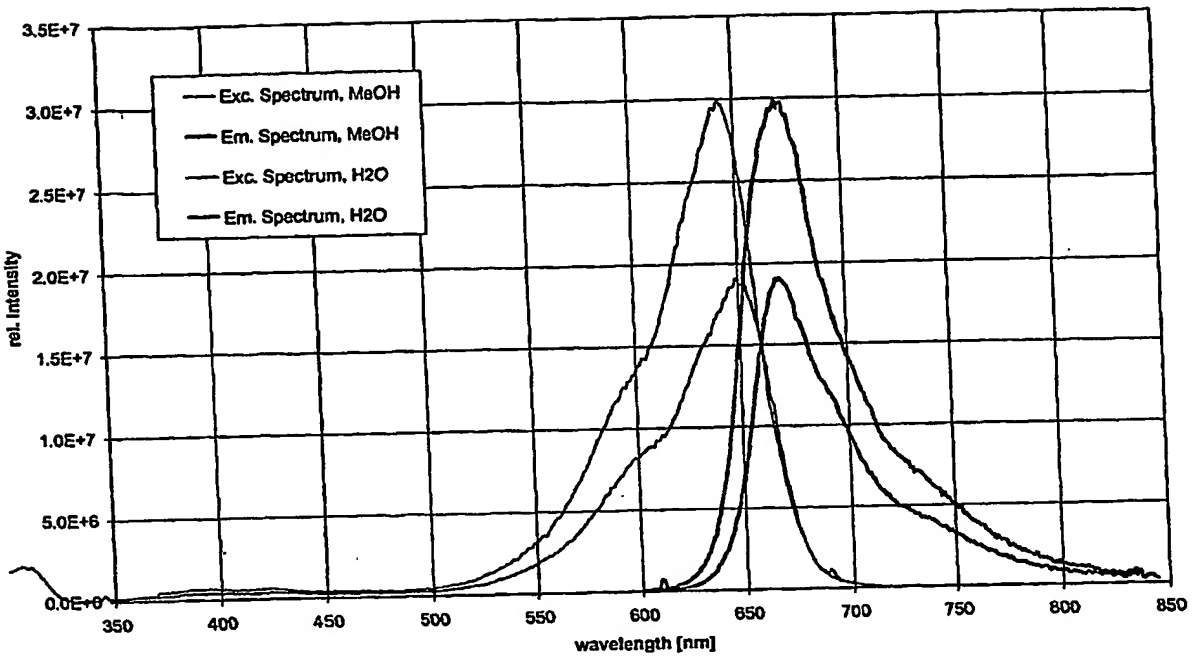
Table 2: Fluorescent properties of compounds of the invention

Compound	solvent	Conc.	Absorption		Fluorescence	
Example		[M/l]	λ_{max} [nm]	ε [l/Mcm]	λ_{max} [nm]	ϕ [%]
Ex. 2	MeOH	1.344E-6	649	67430	677	61
Ex. 2	H_2O	1.706E-6	657	56700	677	24
Ex. 2	mouse serum	1.706E-6	658	-	677	28
Ex. 3	MeOH	1.526E-6	644	64570	670	61
Ex. 3	H_2O	1.531E-6	650	61930	670	37
Ex. 3	mouse serum	1.531E-6	650	-	670	41
Ex. 4	MeOH	6.706E-6	659	13920	695	28
Ex. 4	H_2O	5.828E-6	665	12880	695	12
Ex. 4	mouse serum	5.828E-6	665	-	695	13

Fluorescence Spectra of compound of Example 2 in methanol and water



Fluorescence Spectra of Example 3 in methanol and water



Example 7: Labeling of APP23 mouse and human Alzheimer disease (AD) brain sections using a compound of the invention or Thioflavine S

Four-micrometer thick paraffin sections from an APP23 mouse at 26 months of age are deparaffinized in xylene and rehydrated. 10 mg of the agent of the invention (compound Ex.3) is dissolved in 1 ml DMSO and diluted with deionized water 1:10. This staining solution is applied on sections for about 20 min. Section background is cleared by washing with 95% ethanol. Finally sections are dehydrated in 99% ethanol, cleared in xylene and mounted with Vectashield™. Twenty micrometer thick cryotome sections from a AD brain cortex are air dried and fixated in 4% PFA for 5 min. After washing in tap water sections are stained either with Thioflavine S or with the agent of the invention (compound Ex. 3) for 5 min and further processed as described above. The agent of the invention is dissolved in DMSO and diluted to a final concentration of 0.01 % with 50% Ethanol, Thioflavine S is dissolved in 50% Ethanol, final concentration is 0.01 %.

Example 8: In vivo labeling of Aβ in APP23 mice

Injection solution is prepared fresh by dissolving 10 mg of the agent of invention in 0.2 mL DMSO diluted with 9.8 ml sterile water. Lower concentrations are prepared by further dilution with water. Four APP23 female mice at 21 month of age receive one single injection of the compound (Injection volume: 1 ml/100gr body weight). The treated animals are killed by decapitation after one hour. The brains are removed and frozen on dry ice. 14 µm thick sections are cut in a cryotome, thaw mounted and air-dried. Staining is performed as described above. Sections are analyzed using conventional fluorescence microscopy and confocal microscopy.

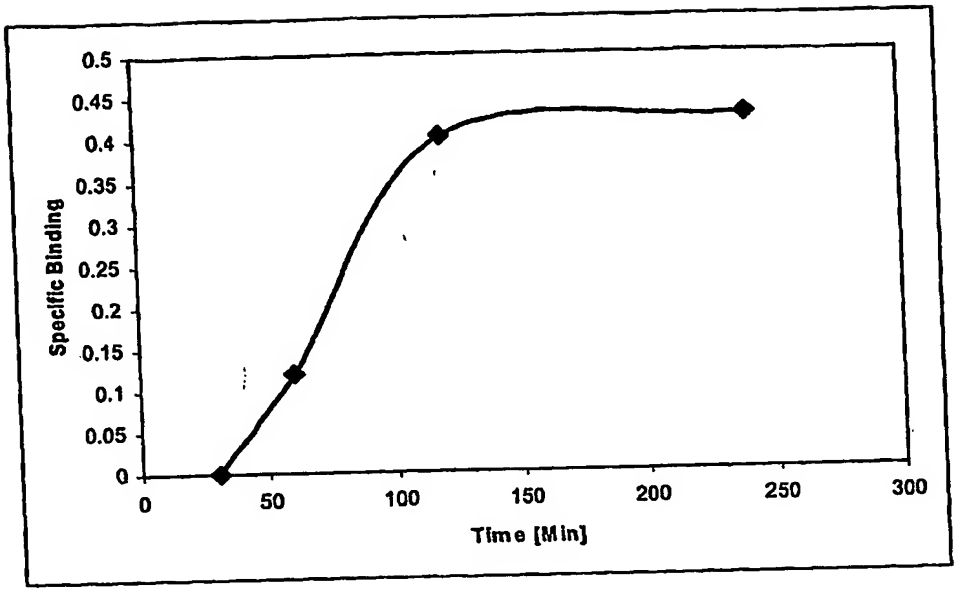
(i) Staining of APP23 mice brain sections (which contain amyloid deposits but no neurofibrillary tangles): The agents of the invention strongly stain amyloid plaques and vascular amyloid deposits in brain sections of APP23 mice.

(ii) Staining of human AD brain sections (which contain both amyloid deposits and neurofibrillary tangles): Brain sections taken from frontal cortex of AD patients are stained with the agents of the invention, and the results compared with a Thioflavine S stain. The agents of the invention intensely and selectively stain amyloid deposits.

(iii) Ex vivo staining in APP23 mice: Intravenous administration of the agents of the invention in APP23 mice leads to a selective and intense staining of amyloid deposits, analyzed ex vivo.

Example 9: Label and real-time detection of beta amyloid plaques and neurofibrillary tangles in vivo using near-infrared imaging

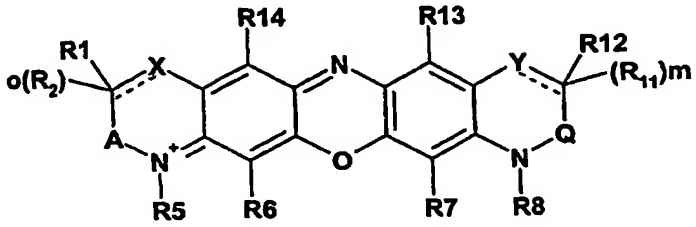
For in vivo near-infrared imaging, 10- to 24-months-old transgenic APP23 mice ($3 \leq n \leq 5$) and as control, non-transgenic APP23 mice of the same age ($3 \leq n \leq 4$) are injected intravenously (i.v.) into the tail-vein with 0.1, 1 and 3 mg/kg agent of invention (0.1, 1 and 3 mg/10 ml in 0.9% saline). Images are recorded 30, 60, 120 and 240 min after systemic administration. The graph shows specific binding of the agent of invention to AD plaques in 16-months-old transgenic APP23 mice that are injected i.v. with 3 mg/kg agent of invention. Here, specific binding is defined as fluorescence signal(transgenic mice) minus fluorescence signal(non-transgenic mice) divided by fluorescence signal(transgenic mice).



Significant differences in fluorescence signal intensity, i.e. specific binding is observed 60, 120 and 240 min after administration of the agent of invention in transgenic APP23 and non-transgenic control mice thus demonstrating the capability of the agent of invention for labeling amyloid plaques in the brain in order to identify Alzheimer's disease.

CLAIMS

1. Compounds of formula I are provided in free base or acid addition salt form



X, Y = C or Heteroatom

X and Y are not simultaneously C;

m, o = 0 or 1, with the proviso that

if m is 0 then the dotted line between Y and the neighbouring C atom represents a bond,

if m is 1 then the dotted line between Y and the neighbouring C atom is absent,

if o is 0 then the dotted line between X and the neighbouring C atom represents a bond,

if o is 1 then the dotted line between X and the neighbouring C atom is absent;

wherein A represents $(CR_3R_4)_p$ and Q represents $(CR_9R_{10})_n$,

n, p = 0 or 1

R6, R7, R13, R14 = H, Hal, (C_{1-4})alkyl, $SO_2(C_{1-4})$alkyl, SO_3H, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy, OH, $NR_{15}R_{16}$

R1, R_2, R3, R4, R9, R10, R_{11}, R12 = H, (C_{1-4})alkyl, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy

or when X = C; R1, R_2 can also be OH, $NR_{15}R_{16}$

or when Y = C; R_{11}, R12 can also be OH, $NR_{15}R_{16}$

R5, R8, R15, R16 = H, (C_{1-4})alkyl, (C_{1-4})alkoxy, (C_{1-4})alkylCOOR$_{17}$, (C_{1-4})alkyl(reactive group)* * as defined in Table 1 and

R17 = H, (C_{1-4})alkyl

2. A compound of formula I according to claim 1 in free base or acid addition salt form wherein X is O,S, or C and Y is O,S, or C with the proviso that X and Y are not both C.

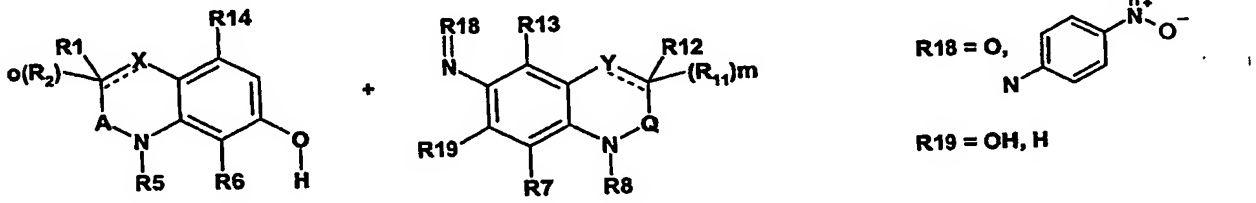
3. A compound of formula I according to claim 1 wherein said compound is 4,8-Dimethyl-2,3,4,9,10,11-hexahydro-1,6-dioxa-4,13-diaza-8-azonia-pentacen chloride (I); 8-Ethyl-4-methyl-2,3,4,9,10,11-hexahydro-1,6-dioxa-4,13-diaza-8-azonia-pentacen chloride (II); 4,8-Dimethyl-3,8,9,10-tetrahydro-2H-1,6,11-trioxa-8,13-diaza-4-azonia-pentacen tetrafluoroborate (III); 4,8-Dimethyl-2,3,9,10-tetrahydro—4H-1,6-dioxa-11-thia-4,13-diaza-8-azonia-pentacen chloride (IV); or 8-(3-Ethoxycarbonyl-propyl)-4-methyl-3,8,9,10-tetrahydro-2H-1,6,11-trioxa-8,13-diaza-4-azonia-pentacen chloride (V).

4. A compound of formula I according to any one of claims 1 to 3 capable of being detected by near-infrared radiation.

5. A composition comprising a compound according to of any one of claims 1-4 and an acceptable excipient or diluent.

6. A composition according to claim 5 capable of being detected by near-infrared radiation.

7. A process for the production of a compound of formula I or a salt thereof, comprising the steps of :



X, Y = C or Heteroatom

X and Y are not simultaneously C;

m, o = 0 or 1, with the proviso that

if m is 0 then the dotted line between Y and the neighbouring C atom represents a bond,

if m is 1 then the dotted line between Y and the neighbouring C atom is absent,

if o is 0 then the dotted line between X and the neighbouring C atom represents a bond,
if o is 1 then the dotted line between X and the neighbouring C atom is absent;

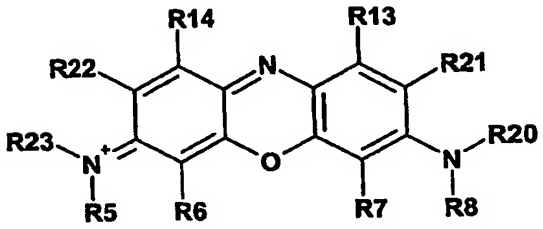
wherein A represents $(CR_3R_4)_p$ and Q represents $(CR_9R_{10})_n$,
n, p = 0 or 1
R6, R7, R13, R14 = H, Hal, (C_{1-4})alkyl, $SO_2(C_{1-4})$alkyl, SO_3H, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy, OH, $NR_{15}R_{16}$
$R1, R_2$, R3, R4, R9, R10, R_{11}, R12 = H, (C_{1-4})alkyl, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy
or when X = C; $R1, R_2$ can also be OH, $NR_{15}R_{16}$
or when Y = C; R_{11}, R12 can also be OH, $NR_{15}R_{16}$
R5, R8, R15, R16 = H, (C_{1-4})alkyl, (C_{1-4})alkoxy, (C_{1-4})alkyl$COOR_{17}$, (C_{1-4})alkyl(reactive group)* * as defined in Table 1 and R17 = H, (C_{1-4})alkyl
and recovering the resulting compound of formula I in free base form or in form of an acid addition salt.

8. A method of labeling target structures in the brain comprising:
(i) applying a composition comprising a compound of formula I according to any one of claims 1-4 in free base or acid addition salt form
(ii) allowing sufficient time for said compound to be chemically associated with the target structure in the brain
(iii) detecting said compound using near-infrared radiation.

9. A method of labeling target structures in the brain comprising:
(i) applying a composition comprising a compound of formula I according to any one of claims 1-4 wherein X is O,S, or C and Y is O,S, or C with the proviso that X and Y are not both C
(ii) allowing sufficient time for said compound to be chemically associated with the target structure in the brain
(iii) detecting said compound using near-infrared radiation.

10. A method of labeling target structures in the brain comprising:

(i) applying a composition according to claim 5 or claim 6 comprising a compound of formula II



formula II

wherein R6, R7, R13, R14 is H, Hal, (C_{1-4})alkyl, $SO_2(C_{1-4})$alkyl, SO_3H, COOH, $COO(C_{1-4})$alkyl, (C_{1-4})alkoxy, OH, $NR_{15}R_{16}$;
R21, R22 is hydrogen,(C_{1-4}) alkyl, (C_{1-4})alkoxy, phenyl, phenylalkyl, COOH, halogen;
R14 together with R22 can form a saturated or unsaturated C bridge,
R21 together with R22 can form a saturated or unsaturated C bridge;
R5, R8, R20, R23 is hydrogen, ,(C_{1-4}) alkyl, (C_{1-4})alkoxy, polyoxyhydrocarbyl, phenyl, phenylalkyl;
R8 together with R20 can form a saturated or unsaturated C bridge,
R23 together with R5 can form a saturated or unsaturated C bridge,
R22 together with R23 can form a saturated or unsaturated C bridge,
R5 together with R6 can form a saturated or unsaturated C bridge,
R7 together with R8 can form a saturated or unsaturated C bridge,
R20 together with R21 can form a saturated or unsaturated C bridge
(ii) allowing sufficient time for said compound to be chemically associated with the target structure in the brain
(iii) detecting said compound using near-infrared radiation.

11. A method according to any one of claims 8-10 wherein said target structures are amyloid plaques.

12. A method according to claim 11 for identifying diseases related to amyloid plaque generation and/or aggregation.

13. A method according to claim 11 or claim 12 for identifying Alzheimer's disease.

14. Use of a compound of formula I according to any one of claims 1-4 in free base or acid addition salt form as a near-infrared imaging agent.

15. Use of a compound of formula I according to any one of claims 1-4 in free base or acid addition salt form wherein X is O,S, or C and Y is O,S, or C with the proviso that X and Y are not both C, as a near-infrared imaging agent.

16. Use of a compound of formula II according to claim 10 as a near-infrared imaging agent.

17. Use according to any one of claims 14-16 as a near-infrared imaging agent to image amyloid plaques.

18. A conjugate comprising a compound of formula I according to any one of claims 1-4 covalently linked to a biomolecule through a reactive group.

19. A conjugate according to claim 18 wherein the biomolecule is selected from the group consisting of: nucleoside, nucleotide, oligonucleotide, nucleic acid, protein, peptide, amino acid, polysaccharide, oligosaccharide, monosaccharide, drug or a small molecule less than MW 500.

20. A conjugate according to claim 18 or claim 19 capable of being detected using near-infrared radiation.